Atty. Docket No.: 7032/2002

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	**Matthew L. Meyerson**
Serial No.:	**09/839,186**
Filed:	**April 19, 2001**
Entitled:	**"Computational Subtraction Method"**

Examiner: Allen

Group Art Unit: 1631

Conf. No.: 1540

#13 Plunkett 11/21/03

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF MATTHEW L. MEYERSON UNDER 37 C.F.R. §1.132

Sir:

I, Matthew L. Meyerson, M.D., Ph.D., hereby declare that:

1. I received a Ph.D. from Harvard University in 1994 and an M.D. degree from {Harvard Medical School in 1993. I am currently an Assistant Professor of Pathology at Harvard Medical School and perform research in the Department of Adult Oncology at the Dana Farber Cancer Institute. My laboratory is focused on identifying and understanding the molecular events that cause human diseases, with a particular concentration on identifying infectious causes for diseases of unknown origin. The primary approach involves the use of computational subtraction. My research publications in this area include the following peer-reviewed publications:

Xu Y, Stange-Thomann N, Weber G, Bo R, Dodge S, David RG, Foley K, Beheshti J, Harris NL, Birren B, Lander ES, and Meyerson M., 2003, "Pathogen discovery from human tissue by sequence-based computational subtraction," Genomics 81(3):329-35.

Weber G, Shendure J, Tanenbaum DM, Church GM, Meyerson M., 2002, "Identification of foreign gene sequences by transcript filtering against the human genome," Nature Genetics 30(2): 131-133.

2. I have read the Office Action mailed in the above-referenced patent application on February 10, 2003, and understand that claims have been rejected for alleged lack of enablement. The Office Action states that

> The specification does not provide databases containing expressed sequence tags (ESTs) or genomic sequences for all host organisms embraced by the claims. While the specification indicates that many such databases are available, there is no evidence that these databases possess the type of sequence integrity (produced from host organisms not having a particular pathogenic condition [citing claim 8]) required by the claims. For example, with respect to claim 2, genome databases for host organisms with known symbiotic organisms would reasonably be expected to contain at least some of the symbiotic sequences. The Relman reference acknowledges that microbial and viral transcripts will be present in what are ostensibly human EST databases. The specification also acknowledges that microbe sequences can be present due to contamination in preparing libraries or sequences rather than actually being present in the host organism.

The Office Action thus concludes that the specification does not describe the claimed invention in such a way as to enable one of skill in the art to perform the claimed methods.

3. In practice, the possible presence of microbial sequences in EST or genomic sequence databases from non-microbial organisms has not interfered with the ability of the claimed methods to determine the presence of microbial sequences in sequences derived from test organisms. In addition to the working embodiments described in Examples 1 and 2 of the specification, I have now successfully used the methods described and claimed in the instant application in another experimental setting, as described herein below.

4. Experiments

In anexperiment employing the approach described and claimed in the present application, non-human transcripts were detected by sequencing cDNA libraries from infected tissue and eliminating those transcripts that match the human genome. A cDNA library was generated from a tissue sample of post-transplant lymphoproliferative disorder (PTLD). 27,840 independent cDNA sequences from the cDNA library were screened by computational subtraction filtering against known human sequences, which identified 32 nonmatching transcripts as candidate microbial sequences. Of these, 22 (0.1%) were found to be amplifiable

from both EBV-infected and noninfected samples and were inferred to be human DNA not yet contained in the available human genome sequence. The remaining 10 sequences could be amplified only from Epstein-Barr virus (EBV)-infected tissues. All 10 corresponded to known EBV sequences. This experiment demonstrates that computational subtraction can detect pathogenic microbes in primary diseased tissue from humans despite the possible presence of microbial sequences in the human sequence databases.

In view of this successful application of embodiments of the claimed invention, it is submitted that the claimed methods are operable to reliably indicate the presence of a microbe inhabiting a host organism, despite the possible presence of microbial sequences in the reference sequence databases from non-microbial organisms. In addition, I note that we have not performed any experiments on diseases of known microbial origin that have failed to detect the causative microbe.

5. The issue of microbial contamination of genomic sequence databases will diminish with the curation and re-sequencing of these databases. At present, the human genomic DNA databases do not contain detectable sequences from pathogenic microbes. Furthermore, the sequences of other microbes can also not be detected in the human genome sequence, to our knowledge. Other genomic sequence databases will rapidly approach the quality of the human genome sequence.

6. Although there does exist contamination of existing EST databases with microbial sequences, these existing databases will not be widely used as the basis for future discoveries.

7. The libraries that we have generated from diseased human tissue have been completely free of microbial sequence contamination, thanks to careful experimental technique. At this point, we have generated over 100,000 unique sequence reads from libraries of human diseased tissue, not known to be cause by pathogenic infection. We have detected no microbial sequences in these libraries.

8. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that wilful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States

Code, and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 9, 2003
Date

Matthew L. Meyerson, M.D., Ph.D.